

AMENDMENTS TO THE SPECIFICATION:

Please replace page 1 of the specification with the following amended page.

Docket No.: GK-ZEI-3156/500343.20157

ARRANGEMENT FOR THE OPTICAL CAPTURE OF EXCITED
AND/OR BACKSCATTERED LIGHT BEAM IN A SAMPLE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority of German application No. 101 60 976.0, filed December 10, 2001, the complete disclosure of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

a) Field of the Invention

The invention is directed to a method in microscopy, particularly fluorescence microscopy, laser scanning microscopy, fluorescence correlation spectroscopy, and nearfield scanning microscopy, for the investigation of predominantly biological specimens, preparations and associated components. This includes methods for screening active ingredients (high throughput screening) based on fluorescence detection. Therefore, simultaneous investigations of specimens with multiple fluorophores in real time by means of simultaneous illumination of the specimen in a plurality of points on the specimen are possible with overlapping fluorescence spectra also in three-dimensional structures of thick specimens.

b) Description of the Related Art

A typical area of application of light microscopy for examining biological preparations is fluorescence microscopy (Pawley, "Handbook of Biological Confocal Microscopy"; Plenum Press 1995). In this case, determined dyes are used for specific labeling of cell parts.

The irradiated photons having a determined energy excite the dye molecules from the ground state to an excited state by the absorption of a photon. This excitation is usually referred to as single-photon absorption (Fig. 1a). The dye

Please replace the paragraph at page 19, line 19 of the specification with the following amended paragraph:

6. Structured line scan – ~~nondescanned~~ nondescanned

Please insert the missing page 20 enclosed herewith.

from the light source LQ must be switched with the beam path from the MDB to the detector DE. Further, the element can also comprise a polarizing element when using polarized excitation light. Its operation is described by way of example with reference to Fig. 7. In this case, the excitation light at MDB is reflected in the HR areas. When using polarized excitation light, polarizing splitters can also be arranged in the HR areas instead of mirrors. Only 50% of the detection light is still lost in the HR area when using polarizing elements.

The arrangements in Figs. 6 to 9 are also suitable for scanning regions of special interest ROI (see EP977069A2. In this case, the laser light is unblocked only for determined regions which are selected beforehand by the user.

The length of the illumination line (along the X-coordinate) can be carried out, for example, by changing the effective focal length of L3 (see Fig. 7, for example). This results in a change in the imaging scale of the microscope arrangement. The change in focal length can be carried out especially quickly in particular by adaptive optics. A further possibility for influencing the line consists in that the line is cut off in ZB1 by an adjustable mechanical diaphragm or two individual knife edges whose width is adjustable. In addition, the position of the line section in the specimen can be influenced by displacing L1 vertical to the optical axis in ZB1, the diaphragm or through the X-scanner. Regions in the specimen which have been optionally defined beforehand by the user can accordingly be acted upon by different excitation light powers.

The advantage of this method compared to the method according to the prior art is that the ROIs can be scanned in real time. By way of example, Fig. 10 shows a ROI in a scan field. The illuminated area is shown in black. The control of the scanners X and Y and diaphragm or MDB is carried out in combination with the element for adjusting the light output corresponding to the ROI to be investigated. The scan field represents the entire section of the specimen that can potentially be investigated by the arrangement.

In principle, the function of the scanners shown herein can also be performed instead by a corresponding scan table (object scanner) at least in one plane.